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A CHEMICAL STUDY OF THE OILS OF SEVERAL
SPECIES OF EUPATORIUM

AND

A STUDY OF THE CHEMICAL AND PHYSICAL
PROPERTIES OF WISCONSIN WORMWOOD OIL

BY

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Chemist to Pharmaceutical Experiment Station*

CONTRIBUTIONS FROM THE COURSE IN PHARMACY

MADISON, WISCONSIN

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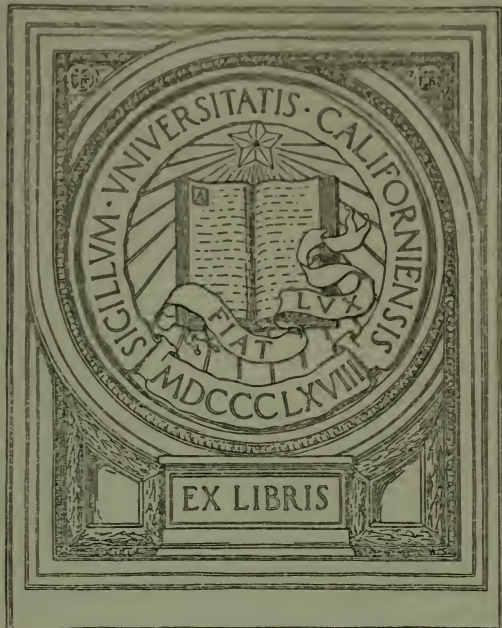
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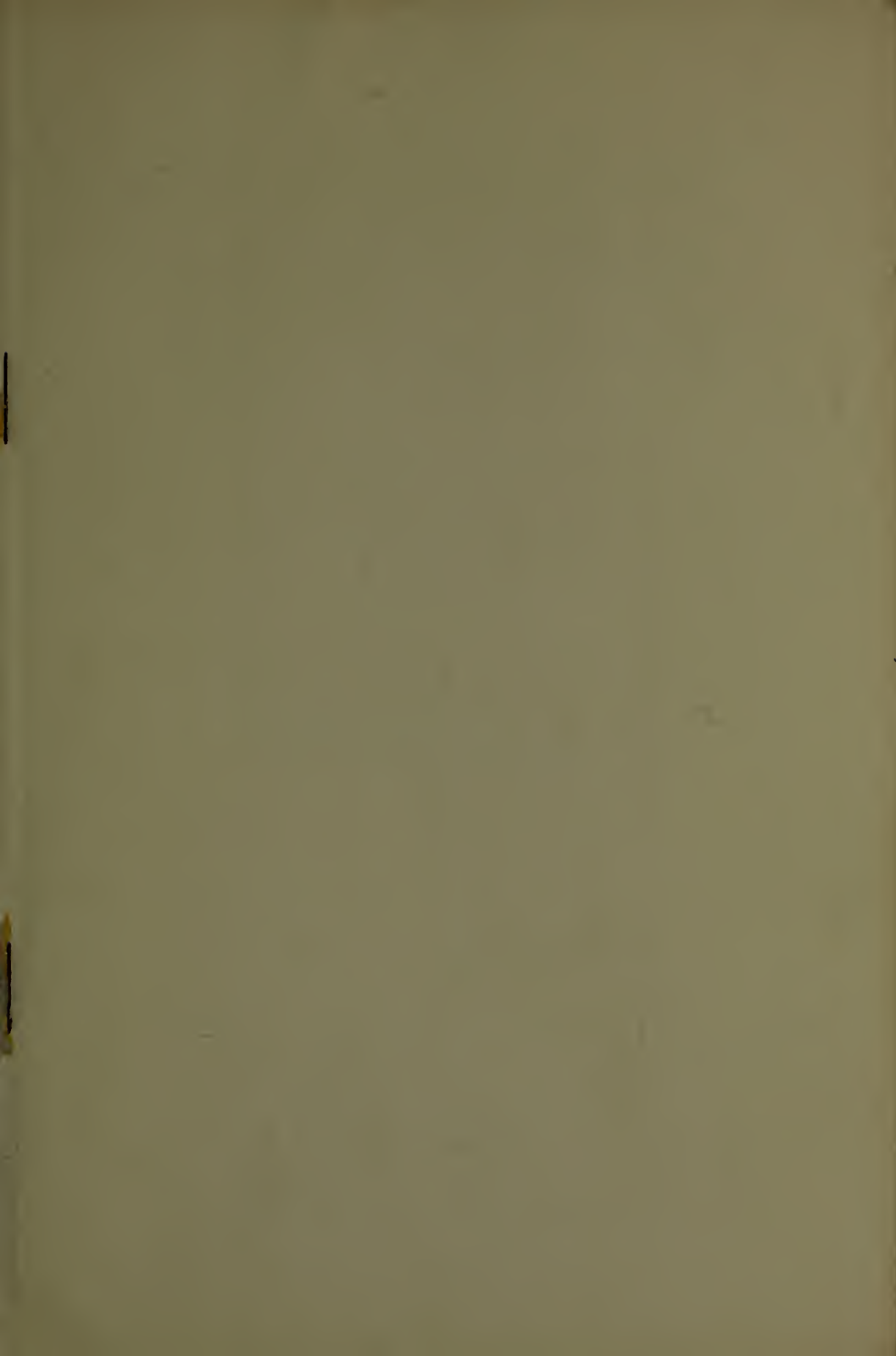
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A CHEMICAL STUDY OF THE OILS OF SEVERAL SPECIES OF EUPATORIUM

The composites to which the genus *Eupatorium* belongs are represented by approximately 12,000 species of which more than 500 belong to the genus under consideration. Of these 500 species probably ten have been examined chemically more or less.

The chemistry of the composites includes a considerable number of representatives of the following phytochemical groups: alkaloids, glucosides, bitter principles, carbohydrates, volatile oils. Yet none of these, save possibly levulin and inulin, can be regarded, even in a measure, as characteristic of the family.

In the more restricted field of the genus *Eupatorium*, which comprises more species than do some of the botanical families, the situation at present is not very different. As already pointed out, only a fraction of one per cent of the known species has been submitted to any chemical examination whatever. Of these, only three (*Eupatorium capilifolium*, *E. triplinerve*, and *E. aromaticum*) have yielded volatile oils thus far. The principal interest in the volatile oils at present centers in the dimethyl ether of hydrothymoquinone which is reported to constitute about 75 to 80 p. c. of the ayapana oil (*E. triplinerve*). Before having been found in ayapana oil, this ether was known to exist only in the oil from arnica root, another composite plant.

Of the 500 or more species of *Eupatorium*, most of which are native to warm and tropical climates, 56 are indigenous to the United States. Although in the warmer regions the genus is represented by a few annuals and shrubs, it is composed in the main of perennial herbs. Some of these grow abundantly, hence invited investigation. Inasmuch as up to the present time no

method has been devised of separating certain phytochemical constituents as conveniently as the volatile constituents are separated by steam distillation, the preliminary chemical survey of the genus was restricted to the study of the volatile oils. For this purpose five species were distilled with the result that two volatile oils were obtained.

As already pointed out one of the principal objects of interest in the chemical study of the genus lay in the discovery of hydrothymoquinone dimethyl ether by Semmler in *E. triplinerve*. This same substance has since been found in *E. capillifolium*. The interest in the chemical study of this genus is enhanced by the medicinal use to which about a dozen of the species are put, principally in this country, but in other countries as well. Several species have been granted official pharmacopoeial recognition (*E. perfoliatum* and *E. purpureum*), but these apparently owe their medicinal value to other constituents than the dimethyl ether referred to, or any other volatile constituents that might find their way into volatile oils.

The following account comprises the results of a chemical investigation of several southern as well as northern species of *Eupatorium* that has extended over about ten years and includes the following species:

Eupatorium capillifolium

E. purpureum

E. hyssopifolium

E. perfoliatum

E. serotinum

EUPATORIUM CAPILLIFOLIUM (LAM.) SMALL. (E. FOENICULACEUM WILD.).

INTRODUCTION

This species grows from Delaware to Florida and Louisiana, from the mountain region to the coast, in low fields, pastures and open woods; but is most abundant in the damp coast plain. It grows also in Cuba and other islands of the West Indies. Under the most favorable conditions for growth it attains a height of about ten feet, but generally is from three to six feet high. It

is paniculately much branched, the leaves are very numerous, mostly alternate, blades much compound, the segments linear-filiform to filiform, corolla white.

It is commonly known as Dog Fennel and Hog Weed. In some localities it is known as Mare's Tail, and in others as Texas Cedar.

According to the *Tallahassee Floridian* (1861) Isaac Bierfield of Newberry, S. C., obtained good results with this plant as a tanning material, but F. P. Porcher¹ states that he examined the plant and found scarcely a trace of tannin in it.²

Culbreth (*Materia Medica*) states that its juice relieves pain from insect bites.

In 1896 Schimmel & Co. reported as follows upon a sample of the oil distilled by Mr. E. Moulié of Jacksonville, Fla.:

"Light yellow color and sharply aromatic, somewhat pepper-like odor, not at all resembling that of the true fennel or anethol. Sp. gr. at 15°C is 0.935, optical rotation in 100 mm. tube is +17°50'. Contains an abundance of phellandrene."

In their report of Nov. 1908 the same firm reports as follows:

"Pale yellow oil from flowering herb, yield about 0.1 p. c.; d_{15}° 0.926; α_D^{20} +18°30'; ester number 7.11. Gives cloudy solution with 3.5 or more volumes of 90 p. c. alcohol. High phellandrene content."

DISTILLATION OF THE OIL

Almost every year since 1903 I have distilled an oil from plants of this species growing in the vicinity of Auburn, Ala. The material first distilled was identified by Professor F. S. Earle.

In all about 30 samples of oil were obtained, the time of collecting the material extending in the main from about the middle of September to the middle of October. Since the oil reported by Schimmel & Co. was dextrorotatory and all my samples were levorotatory, it was decided to distill the plant at various periods to see if a dextrorotatory oil could be obtained.

¹ *Resources of the Southern Fields and Forests*, p. 414.

² An aqueous infusion of the dried plant gives a decided coloration (bluish black) with a solution of ferric chloride.

The first distillation was made May 1st when the plants were about ten inches high. The stems were broken off near the ground and the entire plant distilled. Later when the plants were large a considerable part of the lower portion of the stem was rejected. In such cases the yield of oil would vary considerably by rejecting or including the stems which are heavy and contain but little oil. In the case of large plants the stem was broken off on the average about fifteen inches above the ground. The material was usually distilled in the green state.

In the following table are given the time of collection, the yield of oil, the sp. gr., index of refraction, rotation in a 100 mm. tube, percentage of methoxy group, saponification number, saponification number after acetylating, the acid number and the percentage of thymohydroquinone dimethyl ether calculated from the percentage of methoxyl.

From the table it will be seen that the yield in case of sample No. 1 was 0.36 per cent and that the yield gradually increased to the maximum of 1.11 p. c. in case of sample No. 9, distilled Nov. 16. In several other cases a yield of about 1.35 per cent was obtained but these samples were not studied. The yield of 0.61 p. c. for sample No. 11 was from material collected after one or two light frosts.

Beginning with the sp. gr. 0.9392 in case of oil No. 1 there is a decrease until the minimum 0.9195 is reached in oil No. 4; from this point on there is a gradual increase to 0.96004 in the case of oil No. 11.

The index of refraction for sample No. 1 is 1.504479; there is then a gradual decrease to 1.497808 for sample No. 4. From this there is a gradual increase to 1.506887 for sample No. 10. Then a slight decrease for sample No. 11.

All oils distilled before September have dextrorotation; all distilled in September and after have levorotation. Omitting No. 2 there is a gradual increase in dextrorotation from $+10.27^\circ$ in case of sample No. 1 to $+14.29^\circ$ in the case of sample No. 4. There is then a decrease to $+10.02^\circ$ for sample No. 5 and it is reasonable to suppose that at some time between July 30 and September 8 an optically inactive oil might have been obtained. From No. 6 on there is a rather gradual increase in levorotation.

However, it should be stated here that oils distilled only one day apart have shown as great differences in rotation as that between No. 6 and No. 8.

A glance at the table will show that the percentage of methoxyl is rather high, namely 18.42 for sample No. 1, and then gradually decreases to 14.79 for sample No. 4 and that there is then a gradual increase to 21.45 in the case of sample No. 10, that for No. 11 being slightly lower.

The saponification numbers of the oils, also the saponification numbers after acetylation show an increase reaching the maximum in sample No. 5. Although these values were not determined for all the samples, the data obtained show a diminution in these values for the oils distilled later.

The few acid numbers determined show nothing that would throw light upon the relative composition of the oils.

Table I

When distilled	Yield Percent.	Sp. gr. 25°	$n_D^{27^\circ}$	a_D for 100 mm tube.	D. c. OCH_3	Saponification No.	Sapon. No. after acetylating	Acid No.	D. c. of thymone-hydroquinone dimethyl ether
1 May 1, 1912....	0.36	0.93919	1.504479	+10.27	18.42	5.6	12.94	0.207	57.63
2 May 13, 1912....	0.47	0.9326	1.502330	+7.47	16.87	6.4	13.26	0.206	52.78
3 May 25, 1912....	0.51	0.92378	1.500732	+13.82	15.92	5.38	10.61		49.81
4 June 27, 1912....	0.527	0.91950	1.497808	+14.29	14.79	9.45	43.72		46.02
5 July 30, 1912....	0.567	0.9269	1.49875	+10.02	16.20	16.68	56.85		50.91
6 Sept. 8, 1911....	0.960	0.9278	1.499836	-6.33		13.67		0.18	52.13
7 Sept. 13, 1911....	0.99	0.9346	1.501014	-8.20	16.66	12.26	23.35		
8 Oct. 27, 1911 ...	1.02	0.9472	1.505866	-13.99	20.16			0.25	63.08
9 Nov. 16, 1911....	1.11	0.9505	1.506143	-11.77	20.61	10.53		0.10	64.49
10 Nov. 23, 1911....	1.03	0.9577	1.506887	-11.73	21.45	14.43	30.95		67.11
11 Dec. 3, 1912....	0.613	0.96004	1.506422	-16.70	21.24	10.60	23.83		66.46

PROPERTIES OF THE OIL

The oil prepared from fresh material is generally almost colorless, or of a very light yellow color. That from material which has been dried and kept for some time is usually more highly colored. The odor is somewhat pepper-like but not very sharply aromatic. The yield from fresh material collected in September and early October varied from 0.8 p. c. to 1.35 p. c., being generally above 1 p. c. The optical rotation for oils distilled at the above-named period varied from -3.7° to -16.8° in a 100 mm. tube, though in one case it was as high as -30.1° . The sp. gr. determined at 25°C with water at 25°C as standard varied from 0.9278 to 0.9472; the index of refraction determined at 20°C varied from 1.50055 to 1.50688. Acid numbers obtained for oils distilled in September and October were 0.18 and 0.25 respectively. For oil distilled in September the saponification numbers 10.92, 11.2, and 13.67, respectively, were obtained.

Concentrated nitric acid (70 p. c.) acts energetically upon the oil with production of a reddish brown color. Concentrated sulphuric acid also reacts readily with the oil, producing a similar result. In both cases the degree of color depends upon the relative amounts of oil and acid mixed. Concentrated solutions of alkali carbonates have no apparent effect upon the oil. Cold concentrated aqueous solution of caustic alkali has no apparent effect but on heating produces a yellowish color. With an alcoholic solution of caustic potash the oil is slowly colored, and by heating on the water bath it becomes very dark brown.

Like volatile oils generally, it dissolves readily in ether, chloroform, benzene, acetic ether, and petroleum ether.

With a small amount of alcohol the oil forms a clear mixture, but when more alcohol is added a point is soon reached where the mixture becomes turbid,³ remaining so even after the addi-

³ Other oils are known which show a similar behavior. For instance Kobuschil oil dissolves in 1.2 to 10 volumes of 80 p. c. alcohol but when highly diluted with 80 p. c. alcohol the solution becomes turbid. Gildemeister und Hoffmann, *Die aetherischen Oele*, vol. II, p. 388.

Oil of Champaca flowers is soluble in one volume of 80 p. c. alcohol but when more than 7 volumes of the 80 p. c. alcohol have been added the solution becomes opalescent. The same oil is soluble in 2 volumes of 70 p. c. alcohol but the addition of 4 volumes or more produces decided turbidity. *Ibidem*, II, p. 392.

tion of a large volume of alcohol. It shows the same behavior towards methyl alcohol and glacial acetic acid.

One volume of the oil dissolves in about 0.7 volumes of 90 p. c. alcohol, but when a little more of the alcohol is added it becomes turbid and remains so even after the addition of 50 volumes of the 90 p. c. alcohol.

EXAMINATION FOR ALDEHYDES AND KETONES

With Schiff's reagent the oil gave no reaction, indicating the absence of aldehydes.

Tested with phenyl hydrazine according to directions given by Mulliken⁴ (in test VII—2) there was a very slight opacity.

5 cc. of oil heated with 40 p. c. solution of neutral sodium sulphite in an aldehyde flask gave a slight diminution in volume. From the results it may be stated that ketones are either absent or present in only very slight amount.

EXAMINATION OF THE OIL FOR PHENOLS

In order to determine if phenols were present in appreciable amount 5 cc. of oil were placed in an aldehyde flask, 5 p. c. KOH solution added in small amounts, shaking after each addition, and the flask then filled with water. There was no apparent diminution in the volume of the oil.

The oil was then treated in the usual way for removing phenols, using a 4–5 p. c. solution of potassium hydroxide. There was obtained about 0.11 p. c. of a very thick reddish-brown liquid with a slightly acrid, bitterish taste. With alcoholic ferric chloride there was scarcely any change of color noticeable, but on addition of water a slight purplish color was observed, an indication of the presence of salicylic acid.

IDENTIFICATION OF METHOXYL

In order to determine if a methoxy compound was present some of the original oil was heated with concentrated hydriodic acid according to Perkin's⁵ modification of Zeisel's method.

⁴ *Identification of Pure Organic Compounds*, vol. I, test VII—2.

⁵ *Jour. Chem. Soc.* 1903, p. 1367.

with the exception that the tube leading the liberated methyl iodide to the silver nitrate solution extended below the surface of the liquid. A generating flask with a neck about 16 inches long was used. For one experiment about 12cc. of hydriodic acid and 3cc. of acetic anhydride were used. The flask was heated in a glycerin bath at 140° – 145° C. A blank test gave no precipitate in the silver nitrate solution, showing that both the condenser and vessel containing red phosphorus could safely be omitted. In the quantitative determinations the silver iodide, after evaporation of the alcohol, was transferred to a Gooch crucible, washed and dried to constant weight at 130° C. The percentage of methoxyl found was 16.66 p. c.

In order to remove all doubt as to whether the group present in the oil was the OCH_3 group or the OC_2H_5 group the alkyl halide was passed into an alcoholic solution of dimethyl aniline. After a short time a white crystalline substance separated from the alcoholic solution. After purification by re-crystallization from alcohol this compound had the melting point of 213° – 214° . The melting point of trimethyl phenyl ammonium iodide, the compound which would have been formed in the above experiment if the oil had contained a methoxy group, is 211° – 212° ⁶. If, on the other hand the oil contained the ethoxy group there would have been formed dimethyl ethyl phenyl ammonium iodide which melts at 124.5° – 126° ⁷.

Analyzed for iodine by Stepanow's method⁸ 0.3085 gm. of the substance gave 0.2765 gm. of silver iodide. This corresponds to 48.42 p. c. of iodine. The theoretical amount of iodine for trimethyl phenyl ammonium iodide is 48.23 p. c.

In another experiment the alkyl halide obtained by using the fraction of oil boiling at 244° C. was passed through a condensing tube surrounded by ice. A small amount of a heavy, slightly reddish colored liquid with an odor suggesting methyl iodide collected in the receiver. When distilled it passed over almost completely at 42° – 43° C.

These experiments may be considered proof of the presence of the OCH_3 group and the absence of the OC_2H_5 group.

⁶ Feist, *Ber.* 33, p. 2094, (1900)

⁷ Claus and Howitz, *Ber.* 17, p. 1325 (1884)

⁸ *Ber.* 39, p. 4056 (1906)

FIRST FRACTIONATION OF THE OIL

In 1904 a sample of the oil was freed from phenol, dried and fractionated, without previous saponification, under a pressure of 8–10 mm.

Under the attending circumstances it was not possible to pursue the work far, but notwithstanding its incompleteness the results are recorded here in order to compare them with those of the corresponding fractions of other samples of the oil.

The rotation was determined in a 100 mm. tube at 20°C. in a Laurent polariscope, using sodium light.

Table II

Fraction.	Boiling point.	Rotation.
1.....	Below 58°	+48.2°
2.....	58–65°	+43.5°
3.....	65–75°	
4.....	75–95°	+12.8°
5.....	95–100°	– 0.7°
6.....	100–110°	– 3.7°
7.....	110–115°	–10.5°
8.....	115–120°	–15.9°
9.....	120–125°	–21.5°
10.....	125–130°	–28.5°
11.....	130–135°	–32.0°
12.....	135–143°	–29.0°
13.....	143–160°	– 7.1°
14 Residue.....		

Fraction No. 1. had a sp. gr. of 0.83134 at $\frac{20^\circ}{20^\circ}$

An elementary analysis gave the following results:

0.1754 gm. of the substance gave 0.1844 gm. H_2O = 11.68 p. c. H. and 0.5628 gm. CO_2 = 87.5 p. c. C.

Computed for $\text{C}_{10}\text{H}_{16}$: H = 11.76 and C = 88.23.

The first four fractions together constitute about 15 p. c. by volume of the original oil.

Fraction No. 1. when distilled under ordinary pressure came over at 165° to 170° C.

Fraction No. 8. Sp. gr. = 0.97334 20°/20°. Analysis: 0.1873 gm. gave 0.5201 gm. CO₂ = 75.73 p. c. C and 0.1650 gm. H₂O = 9.78 p. c. H.

Fraction No. 9. Sp. gr. = 0.97543 at 20°/20°. Analysis: 0.2253 gm. gave 0.6194 gm. CO₂ = 74.98 p. c. C and 0.1944 gm. H₂O = 9.59 p. c. H.

Fraction No. 10. Sp. gr. 0.97406 20°/20°. Analysis: 0.2040 gm. gave 0.5718 gm. CO₂ = 76.44 p. c. C and 0.1806 gm. H₂O = 9.83 p. c. H.

Fraction No. 11. Sp. gr. 0.96879 20°/20°. Analysis: 0.2185 gm. gave 0.6184 gm. CO₂ = 77.18 p. c. C and 0.1986 gm. H₂O = 10.09 p. c. H.

Fraction No. 12. Analysis: 0.1430 gm. gave 0.4055 gm. CO₂ = 77.33 p. c. C and 0.1332 gm. H₂O = 10.32 p. c. H.

Fraction No. 13. Analysis: 0.1342 gm. gave 0.3902 gm. CO₂ = 79.30 p. c. C and 0.1372 gm. H₂O = 11.35 p. c. H.

RESIDUE: This residue was very thick and hardened on cooling. To remove it from the flask the following were tried successively; alcohol, absolute alcohol, methyl alcohol, ether, acetone, strong alkali solution, nitric acid, glacial acetic acid,—all without apparent effect. After washing with alcohol it was heated on a water bath with absolute alcohol. This loosened it from the flask but apparently dissolved little or none of it. It was then treated with chloroform in which it dissolved readily. This solution was added to alcohol with stirring. The substance was thrown out of solution, then washed with alcohol and dried. It formed a white, light, odorless, tasteless powder, similar in appearance to light calcined magnesia.

Analysis: 0.1230 gm. gave 0.3796 gm. CO₂ = 84.17 p. c. C and 0.1232 gm. H₂O = 11.12 p. c. H.

Computed for (C₁₀H₁₈)_x, C = 88.23; H = 11.76.

Computed for C₂₀H₃₄O, C = 83.33; H = 11.11.

FRACTIONATION OF THE OIL WITH STEAM

The oil from which the phenols had been removed was then saponified by boiling for one hour on a water bath with about an equal volume of half normal alcoholic potassium hydroxide solution. After washing until alkali was no longer present, 500 cc. of oil were distilled with steam, using as a fractionating

column a plain glass tube three feet long, and about $\frac{3}{4}$ inch internal diameter. Owing to the bumping occasioned by the viscid character of the oil, it was not possible to apply much heat to the flask, consequently it was necessary to discontinue distillation quite often in order to siphon off the water which had condensed in the flask.

In this way there was some loss of oil. The oil drawn off was quite milky even after standing 24 hours. Fractions of 200 cc. each were made and the oil removed from each by means of a separatory funnel. The rate of distillation was about 90 drops per minute. Usually five or six fractions were obtained before discontinuing distillation. The following fractions were obtained, together with a small amount of a thick reddish-brown residue.

The following table shows the amount of oil obtained from each 200 cc. of distillate, with the sp gr., optical rotation and index of refraction for each fraction of oil. As will be noticed from the table the first fraction of oil obtained on renewing the distillation was considerably larger than the one just preceding it.

Table III

No. of fraction.	No. cc. of oil	Density $\frac{25^\circ}{25^\circ}$	α_{D23°	n_{D25°
1.....	59.5	0.84916	+50.35	1.477400
2.....	41.0	0.86629	+42.65	1.482862
3.....	28.0	0.88979	+29.28	1.488762
4.....	20.5	0.91691	+ 9.07	1.496110
5.....	15.7	0.93670	- 6.25	1.501390
6.....	15.1	0.94455	-12.40	1.503082
7.....	15.9	0.94794	-14.84	1.503828
8.....	14.9	0.95179	-17.62	1.504665
9.....	13.5	0.95474	-20.14	1.505222
10.....	12.3	0.95731	-22.75	1.505774
11.....	11.1	0.95945	-24.98	1.506422
12.....	12.0	0.96050	-25.44	1.506701
13.....	11.1	0.96220	-27.28	1.507256
14.....	10.9	0.96376	-29.22	1.507440
15.....	10.5	0.96461	-30.55	1.507716

No. of fraction.	No. cc. of oil	Density $\frac{25^\circ}{25^\circ}$	$\alpha_D^{23^\circ}$	$n_{D_{25^\circ}}$
16.....	10.0	0.96671	—31.56	1.507900
17.....	11.2	0.96681	—31.90	1.508084
18.....	10.0	0.96765	—33.40	1.508268
19.....	9.8	0.96763	—34.96	1.508452
20.....	9.5	0.96837	—35.80	1.508636
21.....	9.2	0.96856	—36.61	1.508636
22.....	9.4	0.96854	—37.28	1.508728
23.....	9.0	0.96837	—38.75	1.508820
24.....	9.5	0.96831	—39.55	1.508820
25.....	8.7	0.96809	—40.75	1.508820
26.....	8.5	0.96791	—41.70	1.508820
27.....	8.5	0.96789	—42.18	1.509096
28.....	8.0	0.96749	—43.07	1.509096
29.....	7.8	0.96645	—45.05	1.509096
30.....	8.0	0.96589	—46.06	1.509096
31.....	7.8	0.96491	—47.90	1.509096
32.....	7.0	0.96340	—49.80	1.509004
33.....	7.0	0.96256	—50.85	1.509004
34.....	5.7	0.96184		1.508452
35.....	5.0	0.96164		1.508268
36.....	3.5	0.96164		1.508084
37.....	2.0	0.96323		1.507256
38.....	1.5			
39.....	1.0			
40.....	0.8	0.968603		1.507348
41.....	0.4			
42.....	0.3			
43.....	0.2			

SEPARATION OF TERPENES BY DISTILLATION WITH ALCOHOL

Since Fendler⁹ succeeded in separating reasonably pure pinene from oil of Cascarilla bark by distillation with alcohol, it was decided to apply the same method to this oil. Accordingly a mix-

⁹ *Arch. der Pharmacie* 238, p. 682.

ture of 500 c. c. of oil and 500 c. c. of 95 p. c. alcohol was distilled on a water bath until about 500 c. c. of distillate were obtained, then 500 c. c. more of alcohol were added to the flask and the distillation continued until another 500 c. c. of distillate were obtained. The alcoholic distillates were separately mixed with a large volume of water, the oil which separated removed and washed with water. This addition of alcohol and distillation was continued in the same manner until the amount of oil removed by one distillation was very small. In obtaining the twelfth fraction absolute alcohol was used; the total volume of distillate was 490 c. c. and the amount of oil separated was 12 c. c., considerably less than that obtained with 95 p. c. alcohol.

In the following table is given the amount of oil separated by each distillation, together with the optical rotation and the index of refraction for each fraction of oil.

Table IV

No. of fraction.	Amt. c. c. of distillate.	Amt. c. c. of oil.	Rotation in 100 mm. tube.	Index of refraction.
1.....	505	27.00	+55.5°	1.477988
2.....	515	21.5	+55.8	1.478184
3.....	520	15.5	+53.6	1.479845
4.....	510	13.5	+52.1	1.480820
5.....	512	10.5	+42.3	1.484220
6.....	505	9.5	+40.2	1.485578
7.....	515	7.7	+35.3	1.488186
8.....	505	6.5	+26.4	1.493640
9.....	500	5.5	+15.01	1.494115
10.....	518	4.5		
11.....	515	3.5		
12.....	490	1.2	-5.03	1.500074
13.....	510	3.5		
14.....	520	3.0		
15.....	510	2.7		

Another 500 c. c. of the same sample of oil were mixed with 500 c. c. of absolute alcohol and distilled as before. In this case

three fractions of oil were obtained, the first two by use of absolute alcohol, the third by using 95 p. c. alcohol.

Table V

Fraction.	Vol. of fraction,	Rotation in 100 m.m. tube.
1.....	14.0 cc	+52.25°
2.....	13.5 "	+53.40°
3.....	18.0 "	+52.30°

From the above tables it will be seen that terpene of a comparatively high degree of purity may be separated from this oil by a single distillation with alcohol. It also seems to be true that a purer terpene can be obtained by using 95 p. c. alcohol. The rotations of the first three fractions obtained by distillation with 95 p. c. alcohol were +55.5°, +55.8°, and +55.63° respectively. When another portion of the same oil was fractionated with steam the first three fractions showed a rotation of +50.35°, +42.65° and +29.28° respectively. From these results it seems that a purer terpene can be separated by alcohol vapor than by steam.

By reference to tables given below it will be seen that repeated fractionation of the oil under diminished pressure gives fractions in which the rotation rises, reaching its maximum in the one case in the second, and in the other case in the third fraction. The same result, though in a lesser degree, is noticed in both distillations with alcohol, while in the case of steam distillation the rotation rapidly diminishes.

FRACTIONATION OF THE OIL

The residue from the 500 c. c. of oil which had been subjected to distillation with alcohol was washed with water, dried with fused sodium sulphate, and together with the fractions obtained by the distillation with alcohol, was fractionated three times under diminished pressure (23 mm.). Then all the fractions boiling above 85° under 23 mm. pressure were fractionated twice at ordinary pressure.

Fourteen fractions and several residues were obtained. The oil used in this fractionation was not saponified. In the following table is given the amount of each fraction, also the optical rotation in 100 mm. tube, the index of refraction at 19°C., and the sp. gr. at 25°C. for each fraction. In the last column is given the percentage of methoxyl in each fraction except the first two which gave practically no reaction for methoxyl. The methoxyl determinations were made by a modification of the Zeisel method described on another page.

Table VI

	Boiling point.	Pressure.	Amt. of fraction. cc.	Rotation at 20° C.	Index of refraction	Sp. gr. $\frac{25^\circ}{25^\circ}$	P. C. of methoxyl.
1	70-72°	23 mm	55	+58.0°	1.4741	0.831	
2	72-75°	"	21	+66.2°	1.4757	0.834	
3	75-85°	"	32	+64.6°	1.4782	0.838	0.99
4	180-190	760	5.5	+49.9°	1.4842	0.872	3.78
5	190-200°	"	7.0	+34.4°	1.4916	0.947	9.26
6	200-210°						
7	210-220°	"	14.5	+ 3.25°	1.5024	0.935	16.09
8	220-225°	"	14.0	- 0.05°	1.5045	0.947	17.96
9	225-230°	"	24.5	- 0.43	1.5050	0.954	20.36
10	230-235°	"	27.5	- 0.20°	1.5054	0.963	21.67
11	235-240°	"	34.0	+ 0.10°	1.5071	0.972	24.00
12	240-245°	"	85.5	+ 1.20°	1.5086	0.977	24.26
13	245-250°	"	15.5	+ 3.50°	1.5077	0.971	22.60
14	250-260°	"	9.0	+ 5.20°	1.5057	0.971	18.58

SAPONIFICATION AND FRACTIONATION OF THE OIL

Since some of the fractions obtained in the first fractionation of the oil were rather small for identification and also for comparison with the results obtained by fractionation with and without previous saponification of oil, 1500 c.c. of oil were boiled an hour and twenty minutes on a water bath with an equal volume of semi-normal alcoholic potassium hydroxide. The mixture was then poured into a large volume of water, the oil separated

and washed, dried with anhydrous sodium sulphate, and then fractionated five times under a pressure ranging from 16 mm. to 25 mm. The following fractions were obtained.

Table VII

Fraction.	Boiling point.	Amt. of fraction.	α_{D27°	$d_{25^\circ}^{25^\circ}$
1	-65°	50 c. c.	+44.10°	0.834
2	65-66°	60 "	+56.98°	0.832
3	66-67°	62 "	+68.92°	0.829
4	67-70°	28 "	+66.70°	0.833
5	70-80°	18 "	+46.18°	0.844
6	80-100°	10 "	+17.85°	
7	100-105°	43 "	-1.07°	
8	105-110°		-1.54°	
9	115-116°	46 "	-1.25°	
10	115-120°	66 "	-1.74°	
11	120-125°	62 "	-4.55°	
12	125-130°	85 "	-5.80°	
13	120-135°	84 "	-8.15°	
14	135-138°	120 "	-11.20°	
15	138-139°	107 "	-14.01°	
16	139-141°	116 "	-20.43°	
17	141-142°	75 "	-28.55°	
18	135°	38 "	-45.54°	
19	135-155°	34 "	-53.50°	
Small residue.....				

With exception of the first six, the above fractions were then distilled once under ordinary pressure. In the following table are given the boiling point, quantity, optical rotation in 100 mm. tube, density at $\frac{25^\circ}{25^\circ}$ and the index of refraction at 29° for each fraction. The percentage of methoxyl is given for four fractions. The amount which distilled below 210° was very small.

Table VIII

Boiling point	Quantity	Rotation in 100 mm. tube	Sp. gr. $\frac{25^\circ}{25^\circ}$	Index of re- fraction	D. c. of OCH_3
210-213°.....	60 c. c.	-1.70°	0.931	1.4970	15.36
213-216°.....	60 "	-1.35°	0.932	1.4981	
216-221°.....	60 "	-1.10°	0.934	1.4986	
221-231°.....	35 "	-0.53°	0.951	1.5028	
231-238°.....	15 "	-0.50°		1.5038	
238-243°.....	60 "	-0.06°	0.976	1.5049	
243-244°.....	120 "	+0.30°	0.934	1.5065	25.34
244°.....	120 "	+0.74°	0.983	1.5067	26.40
244-245°.....	120 "	+1.17°	0.981	1.5065	
245-246°.....	108 "	+1.65°	0.978	1.5058	
246-248°.....	44 "	+2.50°	0.972	1.5054	22.41
248-252°.....	30 "	+3.65°	0.968	1.5048	
252-258°.....		+4.84°	0.961	1.5042	
Residue.....					

LOW BOILING HYDROCARBONS

The physical properties of the first fractions obtained from all the samples of oil fractionated indicate that this portion of the oil consists almost entirely of hydrocarbons. This was proved beyond question by an elementary analysis of fraction No. 1 given in Table II. But in those cases where the oil was repeatedly fractionated the low-boiling fractions have a somewhat lower specific gravity than that of any of the known terpenes. The lowest specific gravity reported for a terpene is probably that for sabinene, namely 0.8400 at 20°C., and that for d- α -phellandrene, 0.8440 at 19°C. The first fraction of oil mentioned in Table II was found to have the specific gravity 0.8313 at 20°C; a very low specific gravity was also found for fraction No. 3, given in Table VII, 0.8295 at 25°C.

In view of these low specific gravities it is possible that some hydrocarbon besides terpene is present in these fractions.

A small amount of one of the unsaturated aliphatic hydro-

carbons, myrcene or ocimene, mixed with the phellandrenes could give specific gravities such as those reported in Table VII for the low boiling fractions. Contradictory to this explanation, however, is the fact that the lowest specific gravities recorded in Table VII, namely 0.8295 coincides with the highest rotation, $+68.92^\circ$. The rotation would be lowered by the presence of myrcene, since it is optically inactive.

With reference to the index of refraction it will be seen that the values recorded for the first three fractions in Table VI, for the first two in Table IV and for the first one in Table III all agree reasonably well with those for the phellandrenes, the lowest recorded in the literature being 1.4732 at 19°C for d- α -phellandrene from elemi oil, the highest being 1.4788 at 20°C for d- β -phellandrene from water fennel oil.

On the other hand the optical rotation for some of the low-boiling fractions recorded in Tables VI and VII is higher than that for any dextro-phellandrene so far reported, the highest being $+60^\circ 21'$ given by Gildemeister and Stephan¹⁰ for a preparation from Schinus oil. The highest rotation reported in this paper is $+80.2^\circ$ for fraction No. 5, Table IX.

IDENTIFICATION OF PHELLANDRENES

Using the method of Wallach,¹¹ nitrosites were prepared from fractions No. 1, 2, 3, and 4, Table VII. The compounds were purified by dissolving them in chloroform and precipitating by means of methyl alcohol. A beautiful soft white mass of silky needles was obtained from each fraction. The yield was small and gradually diminished from fraction No. 1 to No. 4.

The amount of nitrosite obtained from 30 c. c. of each fraction and the melting point of each were as follows:

	Gm.	m. p.
Fraction No. 1	2.52	111–112°
Fraction No. 2	2.22	113–114°
Fraction No. 3	1.98	112–113°
Fraction No. 4 (from 15 c. c.).....	0.92	112–114°

No attempt was made to prepare a nitrosite from fractions No. 5 and 6, but No. 7, when treated in the usual way, gave no

¹⁰ *Arch. der Pharmacie*, 235, p. 591.

¹¹ *Annalen*, 246, p. 282.

nitrosite. The nitrosites were dissolved in chloroform and the optical rotation taken in a 100 mm. tube at 23°C.

Percentage strength of solutions was 10.

Specific gravity of solutions 1.46 at 23°C.

The following values were obtained.

α_{D23}	—40.7° for fraction No. 1.
"	—65.7° for fraction No. 2
"	—76.7° for fraction No. 3.
"	—99.3° for fraction No. 4.

In order to obtain further material for the identification of phellandrenes by the preparation of the nitrosites the low boiling portion of another sample of oil was repeatedly fractionated under a pressure of about 26 mm. The following table gives the boiling point and optical rotation of each fraction, together with the melting point of the nitrosite prepared from each fraction:

Table IX

Fraction	Boiling point	Rotation	m. p. of nitrosite.
1.....	69-70°	+41.0°	100-101°
2.....	70-72°	+47.1°	96- 97°
3.....	72-73°	+68.7°	97- 98°
4.....	73-75°	+72.9°	97- 98°
5.....	75-77°	+80.2°	100-101°
6.....	77-80°	+75.0°	100-101°

In determining these melting points the temperature was raised very slowly. By somewhat more rapid heating they all melted at 112-113°. In optical rotation these nitrosites showed very different results from the first ones prepared. Whereas those first prepared were all levorotatory those from the second sample of oil showed dextrorotation for the first three fractions, and slight levorotation for the fourth. In the last case several readings were taken at intervals of several hours. The rotation gradually diminished from -0.4° to -0.12°. Owing to lack of time the rotation of the nitrosites from the other fractions could not be determined.

Considering the physical properties of the low-boiling portion of this oil and the formation of the nitrosite there can scarcely be any doubt that at least one of the phellandrenes is present.

EXAMINATION FOR PINENE

In the case of every sample of oil which was repeatedly fractionated and even in those cases where the oil was fractionated but once by means of alcohol vapor, fraction No. 1 had a lower dextrorotation than the fractions immediately following. The rotation of fraction No. 1 in the various samples of oil fractionated varied from $+44.1^\circ$ to $+58^\circ$. A small portion of fraction No. 1, table VI was distilled under ordinary pressure. The temperature rose rapidly to about 160°C and a considerable portion of the fraction distilled between 160° and 165°C . The rotation of the distillate was $+54^\circ$ in a 100 mm. tube. The rotation was thus very much reduced by one distillation but owing to evidence of decomposition the fraction was not redistilled. The fact that a part of this fraction distilled between 160° and 165° is positive proof that some hydrocarbon other than a phellandrene is present in the oil. The boiling point of the phellandrenes so far as reported is variously given as 173° to 176°C .

A small amount of dextro- α -pinene mixed with dextro- α -phellandrene would have the effect of lowering the boiling point, the rotation, and the index of refraction and raising the specific gravity.

An attempt was made to prepare a nitrosochloride from the lowest boiling fraction by the method of Wallach but with a negative result.

Inasmuch as the highly optically active pinenes yield little or no nitrosochloride, some of fraction No. 1 of this oil was mixed with an equal volume of a low boiling fraction from oil of *Pycnanthemum Tullia* having a rotation of -44° and this mixture was treated in the usual way for the preparation of the nitrosochloride. The result here was also negative.

So far as boiling point is concerned β -pinene might be present in fraction No. 1, but it can be excluded on the ground of its higher specific gravity, namely 0.8675.

EXAMINATION FOR SABINENE

For this hydrocarbon the following constants have been reported: b. p. 162° – 166° ; sp. gr. at 20°C 0.840 to 0.842; rotation in a 100 mm. tube $+63$ to $+67.5^{\circ}$; index of refraction 1.4660 to 1.4678. These constants agree fairly well with those of fraction No. 1 of the oil under investigation here, except that the rotation given for sabinene is considerably higher, that is almost 20° higher than the lowest rotation obtained for fraction No. 1, table VII. However, results obtained by Schimmel & Co.,¹² in the fractionation of a larger amount of sabinene from oil of savin may be considered a very strong indication of the presence of sabinene in fraction No. 1 of this oil. 20 p. c. of the sabinene in question boiled between 162° and 163° , had the sp. gr. 0.8481 at 15°C , and the rotation in a 100 mm. tube $+59^{\circ}30'$.

EXAMINATION FOR LINALOOL

Fractions boiling above 190°C were repeatedly fractionated, first under diminished pressure and then under ordinary pressure.

A few c. c. of oil were obtained between 195° and 206°C . In order to determine if linalool were present this fraction was oxidized with chromic acid mixture; almost immediately an odor suggesting that of oil of lemon was developed. After shaking the mixture thirty minutes it was made slightly alkaline and then subjected to steam distillation. A pleasant smelling, light yellow oil was obtained. Judging from the odor that citral was probably present as the result of oxidation, an attempt was made to prepare the semicarbazone by the method of Tiemann.¹³ 5 c. c. of the fraction dissolved in 30 c. c. of glacial acetic acid were mixed with 4 gms. of semicarbazide hydrochloride dissolved in a little water. Almost immediately a white solid separated. This was filtered out and found to be soluble in water. Washed with methyl alcohol and dried it was found to have the m. p. 169 – 170° with decomposition. Semicarbazide hydrochloride and

¹² Gildemeister u. Hoffman, *Die aetherischen Oele*, vol. II, p. 301.

¹³ *Ber.* 31, p. 3331; and 32, p. 115.

glacial acetic acid alone gave a white precipitate which melted with decomposition at 170°C . The white compound obtained from the oxidation product of the fraction boiling at $195\text{--}200^{\circ}\text{C}$ was, therefore, not a semicarbazone but unchanged semicarbazide hydrochloride.

Another portion of the oxidized fraction was treated with cyanacetic acid by the method of Tiemann¹⁴ for the preparation of citralidenecyanacetic acid but no condensation product could be separated.

With Schiff's reagent the oxidation product behaved almost exactly like citral. In neither case was a pink color produced, in fact no coloration was produced at once, but a purple color was slowly formed.

A further test was applied by heating 5 c. c. of the oxidized fraction with 40 p. c. solution of neutral sodium sulphite on a water bath. The reaction of the mixture and a slight diminution in volume of the oil indicated the presence of a small amount of aldehyde or ketone. Although the evidence is rather slight it is, nevertheless, considered quite probable that the oil contains a small amount of linalool. All fractions boiling between 195° and 220° yield by oxidation oils which have an odor suggestive of citral. The color of the products from the lower boiling fractions is light yellow, the color gradually becomes darker with the higher boiling products and finally is deep red, due to the presence of thymoquinone which results from the oxidation of thymohydroquinone dimethyl ether.

IDENTIFICATION OF BORNEOL

The fraction boiling at $206^{\circ}\text{--}208^{\circ}$ was oxidized with chromic acid mixture in the same manner as was the fraction boiling at $195^{\circ}\text{--}200^{\circ}\text{C}$. This product was then treated by the method of Emil Fischer for the preparation of camphoroxime. The product after re-crystallization from dilute alcohol was almost colorless. It melted at 116° to 117°C . The m. p. of camphoroxime is given as $117^{\circ}\text{--}119^{\circ}$. The m. p. and odor of the product obtained are in agreement with those of camphoroxime. To de-

¹⁴ Ber. 31 p. 3329.

termine if the oxime obtained might have resulted from camphor present as such and which might have escaped oxidation, some of the original fraction was treated with hydroxylamine hydrochloride in the same manner as the oxidized fraction. No oxime could be separated, though the odor of the mixture suggested that traces of camphor might be present.

The fraction boiling at 208° – 212° was oxidized in the same manner as was the fraction boiling at 206° – 208° and an oxime prepared from the product. The oxime obtained had about the same melting point as that obtained from the preceding fraction, but the yield was larger as might be expected, since the boiling point of borneol is 210° – 212° C.

From the oxidation product of this fraction a semicarbazone was prepared as follows: 2 gm. of semicarbazide hydrochloride, 2 gm. of potassium acetate and 6 c. c. of water were mixed with 12 c. c. of the oxidized liquid and allowed to stand about three weeks. A small amount of a slightly yellowish substance separated which melted at about 201° C. Camphor semicarbazone melts at 236° – 238° , thymoquinone semicarbazone at about 193° C. The substance obtained was evidently a mixture of the two semicarbazones mentioned.

To learn more about the composition of this fraction it was acetylated and the acetylated oil saponified. The acetylation was carried out in the usual manner, the mixture being boiled gently during one hour. In saponification the liquid was boiled on a water bath one hour. A saponification number of 64.6 was obtained. Estimated as an alcohol of the composition $C_{10}H_{18}O$, this would correspond to about 18.67 p. c. alcohol. The small yield of camphor oxime obtained as stated above goes to show that some other alcohol besides borneol is present.

This portion of the oil was fractionated so often it was thought there could not be more than traces of the dimethyl ether of thymohydroquinone present. However, a methoxy determination was made to throw light upon this point. 0.7100 gm. of the fraction gave by the usual method 0.7428 gm. AgI, corresponding to 13.8 p. c. OCH_3 which is equivalent to 43.19 p. c. of thymohydroquinone dimethyl ether. This would leave a considerable part of the fraction unexplained as to composition.

In order to determine if hydrocarbons might still be present an elementary analysis of the fraction was made.

0.3324 gm. of the substance gave 0.2773 gm. H_2O and 0.9444 gm. of CO_2 corresponding to 9.26 p. c. H and 77.48 p. c. C. This result makes it very improbable that a hydrocarbon in appreciable amounts could be present. And thus the composition of a considerable portion of the fraction still remains unexplained. If an alcohol which is decomposed by long boiling with acetic anhydride, such as linalool, be present this would possibly account for the remaining portion of the oil.

This fraction shows a very characteristic reaction with concentrated nitric acid. When treated with a small amount of concentrated nitric acid it is decomposed with the formation of a blue or bluish-green liquid, the color of which, if the right amount of nitric acid is used, persists for a long time when exposed to the air. This reaction was tried with linalool, terpineol and borneol each by itself and also in solution in thymohydroquinone dimethyl ether, but no color similar to that of the fraction of oil could be obtained. It is, therefore, quite evident that there is in this fraction some compound still unaccounted for and that at least one alcohol besides borneol is present.

Fraction $220^\circ\text{--}225^\circ$. Some of this fraction was acetylated and then saponified, whereby a saponification number of 18.26 was obtained which corresponds to 5.09 p. c. of alcohol of the composition $\text{C}_{10}\text{H}_{18}\text{O}$.

An elementary analysis gave the following result: 0.2696 gm. of substance gave 0.2362 gm. H_2O and 0.7724 gm. of CO_2 . Found $\text{C}=78.13$, $\text{H}=9.73$. Computed for an alcohol of the formula $\text{C}_{10}\text{H}_{18}\text{O}$: $\text{C}=77.92$, $\text{H}=11.68$.

Computed for the dimethyl ether of thymohydroquinone, $\text{C}_{12}\text{H}_{18}\text{O}_2$: $\text{C}=74.22$, $\text{H}=9.27$. Since borneol was identified in the fraction boiling at $208^\circ\text{--}212^\circ$ and since the main constituent of the oil quantitatively, is the dimethyl ether of thymohydroquinone which boils at about 245°C it is quite certain that both of these compounds are present in this fraction, the latter predominating. But the high percentage of carbon makes it certain that some compound with a higher carbon content is present.

IDENTIFICATION OF THYMOHYDROQUINONE DIMETHYL ETHER

The physical and chemical properties of the high boiling portions of the oil, especially the fraction boiling at 244°C. suggested the presence of methyl eugenol. Accordingly a portion of this fraction was oxidized with potassium permanganate as follows: About 30 c. c. of the fraction were heated in a large flask with about 1200 c. c. water to 50°C. on a water bath, and to this 24 gm. of KMnO_4 were added in small portions, with thorough shaking. When the liquid was decolorized it was filtered, the filtrate reserved, the precipitate, which still contained unchanged oil, was returned to the flask, mixed with 600 c. c. water, and 12 gm. more of KMnO_4 were then added in the same manner as before. This mixture was filtered, the filtrate united with the first one and evaporated on a water bath to a small volume. The liquid was then acidified and shaken out with ether. After distilling off the ether there remained a small amount of a thick, syrupy mass, with a slight odor of valerianic acid. After long standing no crystals were formed. The next higher boiling fraction was subjected to the same treatment, but with the same result.

The above described treatment was successfully applied by Petersen¹⁵ to a high boiling portion of the oil of *Asarum europaeum* for the identification of methyl eugenol by conversion of the latter into veratric acid. It was also successfully applied by me to a high boiling fraction of the oil of *Asarum arifolium*¹⁶ for the identification of methyl eugenol.

In a further attempt to identify methyl eugenol in this oil, a bromination test which had yielded the tribromderivative of methyl eugenol when applied to a high boiling fraction of the oil of *Asarum arifolium*¹⁷ was also applied here as follows:

About 8 gm. of the fraction boiling at 244°C. were dissolved in chloroform. To this solution, kept cold in a freezing mixture, a chloroformic solution of bromine was added drop by drop, with constant shaking, until a slight excess of bromine had been added. After evaporation of the chloroform by a current of

¹⁵ *Arch. d. Pharm.* 1888, 113.

¹⁶ *Ibid.* 240, 380.

¹⁷ *Ibid.* 240, 382.

dry air, a thick brown colored mass remained from which no crystals could be obtained even after long standing. The same test was applied to the next higher boiling fraction, but with the same negative result. From these results it was concluded that methyl eugenol is not a constituent of the oil.

After all attempts to establish the presence of methyl eugenol had failed, the only other known methyl ether, namely the dimethyl ether of thymohydroquinone, which agrees reasonably well in physical and chemical properties with the fraction boiling at 244°C., was taken into consideration.

The residues obtained by heating the fraction with concentrated hydriodic acid in order to establish the nature of the alk-oxy group present, were not examined until after the absence of methyl eugenol had been reasonably well proved. When these were examined the nature of the ether present was at once shown. After standing a short time a solid body had formed in these acid residues. This was filtered out and washed with water in which it was found to be almost insoluble. The substance was thus obtained almost white. As it was found to be quite soluble in boiling water it was recrystallized from that solvent. It melted at 142°C. According to Carstanjen¹⁸ thymohydroquinone melts at 139.5°C., and according to Ciamician and Silber¹⁹ it melts at 143°C.

When oxidized with chromic acid mixture it yielded a reddish yellow substance of irritating odor, almost insoluble in water, readily soluble in alcohol and having the melting point 44°–45°C. In all these characteristics it agrees perfectly with thymoquinone which results upon oxidation of thymohydroquinone.

It is thus seen that the test which proved the alk-oxy group to be methoxyl, at the same time proved that the high boiling methyl ether present is the dimethyl ether of thymohydroquinone.

Further proof of the correctness of this conclusion was obtained by oxidation of the high boiling fractions. The portion of the oil boiling in the neighborhood of 200°C was oxidized with chromic acid mixture, in the first place, in order to ascer-

¹⁸ *Jour. f. prakt. Chem.* II, 3(1871), 54.

¹⁹ *Atti della Reale Accademia dei Lincei Rendiconti* (5) 10, 1(1901) 96.

tain the nature of the alcohol present. The liquid thus obtained was of a light yellow color characteristic of oil of lemon. All the fractions boiling above 200°C . were next oxidized in the same way; and it was found that there was a gradual change in the color of the products obtained, passing from the light yellow of fraction 200° – 210° to a deep red for all fractions above 238°C . Their odor was somewhat sharp and penetrating, suggestive of thymoquinone.

Several of these red oily products were added to a relatively large volume of water and after saturation with sulphur dioxide the mixture was warmed and violently shaken. After standing several days the color of the oils had changed to a light yellow and a white solid had separated. This was filtered out, recrystallized from boiling water and dried. It melted at 141° – 142°C .

In endeavoring to identify the alcohols present, by oxidation and the formation of a semicarbazone from the oxidation product, it was observed that after standing a short time in the presence of semicarbazide hydrochloride the oxidation product from the fraction boiling at 220° – 230°C gave a precipitate of a bright yellow color. After recrystallization from methyl alcohol the compound melted at 190°C . The red liquid obtained from several other fractions was treated in the same way. All of them gave this yellow compound. In one case the melting point of the compound was 191° – 192°C . and in another 194° – 195°C . Since it was suspected that the yellow compound thus obtained was the semicarbazone of thymoquinone, a small amount of a compound known to be thymoquinone was treated in the usual way with an amount of semicarbazide hydrochloride slightly in excess of the theoretical amount necessary to form the disemicarbazone. The product obtained had the same color and, so far as examined, the same solubilities as the yellow substance obtained from the high boiling fractions of the oil. It melted at about 191°C . After recrystallization from methyl alcohol it melted at 192° – 193°C .

In 1873 Sigel²⁰ found the dimethyl ether of thymohydroquinone in the oil of the root of *Arnica montana* and by oxidiz-

²⁰ *Annalen*, 170, 358–359.

ing with chromic acid mixture the fraction boiling at 224°–245°C. obtained thymoquinone.

Some of the red oxidation product was heated with a saturated solution of normal potassium sulphite to 60°–70°C. and thoroughly shaken. After standing a short time the oil was completely decolorized and a white substance had separated. This was removed and boiled with hydrochloric acid but no thymohydroquinone was obtained.

According to Carstanajen²¹ thymoquinone and normal potassium sulphite yield a potassium sulphonate compound which is decomposed by boiling hydrochloric acid into thymohydroquinone and sulphuric acid.

So far the dimethyl ether of thymohydroquinone has been found in only two other oils. In 1873 Sigel²² reported having identified it in the root oil of *Arnica montana*, about four-fifths of which seemed to consist of this ether. Recently Semmler²³ reported this ether as a constituent of the oil of *Eupatorium triplinerve* (Ayapana oil), apparently constituting about three fourths of the oil.

IDENTIFICATION OF ACIDS

EXPERIMENT NO. 1.

The alkaline aqueous solution resulting from the saponification of two liters of oil was shaken out with ether in order to remove the small amount of non-saponifiable oil present, then concentrated to a small volume, made acid with sulphuric acid and subjected to steam distillation, the process being continued until practically all the volatile acids had been driven over. The distillate was collected in five fractions, of which the first three, especially, possessed an odor suggesting one or more fatty acids, possibly caproic, caprylic or capric acid, or a mixture of these. All the fractions were thoroughly shaken with an excess of barium carbonate, the latter removed, and silver nitrate solution added to the filtrate as long as a precipitate was formed. The

²¹ *Jour. f. prakt. Chem.* II, 15, 480.

²² *Annalen*, 170, 358–359.

²³ *Ber.* 41, 509–512.

silver salts were filtered out, washed and dried at 100°C. The silver salt from the first fractions darkened considerably on drying, the color diminishing gradually from fraction 1 to fraction 5.

ANALYSIS OF THE SILVER SALTS

Fraction 1. 0.1217gm. of the silver salt left on ignition 0.0643 gm. of metallic silver, corresponding to 52.83% of silver.

Fraction 2. 0.2879gm. of the silver salt left on ignition 0.1685gm. of metallic silver, corresponding to 58.52% of silver.

Fraction 3. 0.2514gm. of the silver salt left on ignition 0.1597gm. of metallic silver, corresponding to 63.52% of silver.

Fraction 4. 0.4014gm. of the silver salt gave by ignition 0.2586gm. of metallic silver, corresponding to 64.42% of silver.

Fraction 5. 0.2643gm. of the silver salt gave on ignition 0.1706 gm. of metallic silver, corresponding to 64.54% of silver.

Silver salt of acetic acid contains 64.64% of silver.

Silver salt of butyric acid contains 55.34% of silver.

Silver salt of valerianic acid contains 51.64% of silver.

Silver salt of caproic acid contains 48.39% of silver.

Silver salt of caprylic acid contains 42.59% of silver.

Silver salt of capric acid contains 38.67% of silver.

From the analytical data obtained it follows that the acid present in fractions No. 4 and No. 5 was almost pure acetic acid, and that in fraction No. 3 acetic acid largely predominated. The presence of acetic acid in fractions No. 4 and No. 5 was further proved by the fact that when small amounts of the silver salt from these fractions were heated with a little ethyl alcohol and a little sulphuric acid the odor of ethyl acetate was developed.

The percentages of silver obtained in the case of fractions No. 1 and No. 2 show there was certainly at least one other acid besides acetic acid present in the original mixture, and in fact one having a higher molecular weight than that of acetic acid. But so far as the analytical results obtained are concerned it cannot be stated definitely what other acid or acids may have been present.

EXPERIMENT NO. 2

The aqueous alkaline liquid resulting from the saponification of another sample of the oil was distilled with steam to remove unchanged oil; enough dilute sulphuric acid was then added to neutralize the KOH present and liberate a part of the fatty acid. This mixture was then shaken out with ether, and the ethereal solution shaken several times with 5 per cent solution of sodium carbonate. In this way the fatty acid was separated from the small amount of phenol which may have been liberated at the same time. The sodium carbonate solution was then made acid with dilute sulphuric acid and the liquid distilled with steam, the distillate being collected in six fractions.

Fraction No. 1 consisted of a turbid liquid with a small amount of a slightly yellowish oily liquid floating on top. The odor was somewhat like that of caproic acid. The oily layer was separated and converted into the barium salt from which the silver salt was prepared. Of the dried salt there was only 0.0669gm. On ignition this yielded 0.0275gm. of metallic silver, corresponding 41.1 per cent of silver. This result would indicate the presence of capric acid mixed with a small amount of some lower fatty acid.

The aqueous portion of distillate No. 1 was treated with barium carbonate, filtered, and from the filtrate the silver salt prepared. 0.1103gm. of salt dried at about 100°C gave on ignition 0.0563gm. of metallic silver corresponding to 51.04 per cent of silver. This result agrees fairly well with the theoretical amount of silver in the silver salt of valerianic acid, though the same result might be obtained from a mixture of lower and higher fatty acids.

From the remaining fractions of distillate almost no silver compound could be prepared.

The liquid which had been partially acidified with sulphuric acid and shaken out with ether was now made slightly acid with dilute sulphuric acid and distilled with steam. Five fractions of distillate were obtained, each having a decidedly acid reaction.

Fraction No. 1. 0.1601gm. of silver salt prepared from this

fraction gave on ignition 0.083gm. of metallic silver, corresponding to 51.84 per cent of silver.

Fraction No. 2. 0.1601gm. of silver salt gave on ignition 0.0778gm. of metallic silver, corresponding to 64.24 per cent of silver.

Fraction No. 5. 0.1062gm. of silver salt gave 0.0698gm. of metallic silver, corresponding to 65.72 per cent of silver.

The non volatile acids were not identified.

EUPATORIUM PURPUREUM

This plant, which is most commonly known as Queen of the Meadow, Joe-Pye Weed, Gravel Root, etc., grows quite widely distributed throughout North America. It is one of the largest species of the genus, sometimes growing 8 or 9 feet high. Its flowers are purple, pink, or sometimes nearly white. Its leaves are arranged in whorls of 3 to 6.

As a popular remedy it has been held in high esteem for many years and was accorded official recognition by the United States *Pharmacopoeia* of 1830, New York edition. It still seems to be considered a valuable remedy by physicians of the Eclectic School of medicine. The root is the part used.

To what compound or compounds such medicinal properties as it may possess are due is not known. Although it has been the subject of more or less chemical investigation the only substance which has been studied to any extent is the yellow crystalline neutral principle, *euparin*, discovered by J. U. Lloyd,²⁴ and further investigated by C. C. Manger.²⁵

As the peculiar odor of the flowers suggested the possibility of obtaining from them an interesting volatile oil, about 40 pounds of the fresh material, consisting of leaves and flowering tops were distilled with steam. The material was collected near Madison, on the University Farm.

The distillation was continued about an hour and a half, with the result that no appreciable amount of oil separated from the distillate, which, however, was somewhat turbid and possessed a rather disagreeable odor not unlike that of the flowers.

²⁴ *Am. Journ. Pharm.* 1890, 76.

²⁵ *Ibid.* 1804.

There were indications that the volatile principle is capable of producing decided physiological effects when inhaled.

EUPATORIUM HYSSOPIFOLIUM

This is one of the smallest of the American species of Eupatorium, seldom growing more than three feet high. It is found widely distributed over the eastern part of the United States from Massachusetts to Alabama and Mississippi, growing in dry, sterile soil. It is readily distinguished from all other species of this genus by its opposite, narrowly linear leaves, often crowded in the axils.

In some localities it is considered an antidote to the poisonous bites of reptiles and stings of insects. No chemical examination, it seems, has ever been made of this plant, though its extremely bitter taste suggests the presence of interesting compounds.

In order to learn if it contained volatile oil, about 70 pounds of the herb were subjected to steam distillation. The material was collected near Auburn, Ala., about the middle of September, 1913, and distilled about a month later at the University of Wisconsin.

No oil separated from the distillate.

EUPATORIUM PERFOLIATUM

Of all our native plants this has probably had the most extensive and frequent use as a domestic remedy. Under such names as Boneset, Ague-weed and Feverwort it has been in common use from New Brunswick to Florida, Louisiana, and the Dakotas. The leaves and flowering tops have been official in the *United States Pharmacopoeia* since 1830, but it is rarely prescribed by physicians.

It grows in marshy places on the borders of lakes, ponds, and streams. The stem is from 1 to 5 feet high. The most distinctive feature of the plant is the character of its leaves, which are connate-perfoliate, each pair resembling a single leaf perforated by the stem.

Although several reports have been made concerning the chemical constituents of this plant little is known with certainty.

M. H. Bickley²⁶ distilled a small amount of the leaves and flowers but obtained only a "slightly milky odorous distillate". H. B. Parsons²⁷ made a proximate analysis of the plant and reported among other things traces of volatile oil. Geo. Latin²⁸ reported a glucoside (eupatorin) and a "small quantity of volatile oil having the disagreeable odor of boiled cabbage." H. F. Kaercher²⁹ reported finding about 5 per cent of inulin in the root. A complete proximate analysis was made by C. A. Walter³⁰ in 1900. He reports the important constituents to be (1) a coloring principle, $C_{27}H_{30}O_{17}$, (2) a tannin, $C_{12}H_{18}O_7$, of glucosidal nature, (3) a bitter principle, $C_{35}H_{58}NO_{10}$.

In order to learn definitely how much volatile oil might be obtained from the plant about 30 pounds of the leaves and flowering tops were collected on the University farm and subjected at once to steam distillation. There was obtained a small amount (about 10 minims) of a bluish green oil, lighter than water and of a rather pleasant odor. Judging from this yield it would require about three fourths of a ton of the fresh material to yield one fluid ounce of the oil, though it is quite probable a larger yield would be obtained by cohobating the distillate and working on a larger scale.

THE VOLATILE OIL OF EUPATORIUM SEROTINUM

This plant is indigenous to the eastern part of the United States, its range being from Delaware to Florida, Texas, Kansas and Minnesota. It grows in low alluvial ground, the stem being from three to six feet high. No use seems to have been made of this plant, either in medicine or otherwise.

The material used in the preparation of this volatile oil was collected near Auburn, Ala., about the middle of September 1913. After being dried it was shipped to Madison, Wis., where, about a month later, it was distilled with steam. The weight of the crude material in the fresh condition was about 55 pounds. Practically the whole plant above ground was collected while in blos-

²⁶ *Am. Jour. Pharm.* 26, 495.

²⁷ *Am. Jour. Pharm.* 1879, 342.

²⁸ *Am. Jour. Pharm.* 1880, 394.

²⁹ *Am. Jour. Pharm.* 1892, 511.

³⁰ *Proceedings A. Ph. A.* 1900, 216-232.

som, the flowers being apparently the only part of the plant possessing aromatic properties.

The oil is unusually difficult to separate, which is shown by that fact that it required about 40 hours of continuous distillation to exhaust the material. The distillate was cohobated, but only a comparatively small amount of oil was obtained. The total amount of oil separated was 128 gm. or about 0.51 per cent based upon the weight of undried material.

PHYSICAL PROPERTIES OF THE OIL

When first distilled the oil was colorless, but upon exposure to air and light soon became highly colored reddish brown. The odor was very similar to that of the flowers, the taste slightly warm and bitterish.

One cubic centimeter of the oil became turbid by the addition of two or three drops of 90 per cent alcohol. By the further addition of about 0.5 c. c. of 90 per cent alcohol the mixture became clear. A further addition of 1.7 c. c. of 90 per cent alcohol caused the mixture to become turbid again. The turbidity was again removed by the further addition of 1.8 c. c. of 90 per cent alcohol. 1 c. c. of the oil, mixed with 100 c. c. of 70 per cent alcohol formed a liquid which was still slightly turbid. The mixture of 1 c. c. of the oil and 32 c. c. of 80 per cent alcohol was still somewhat turbid.

The specific gravity of the oil at 25°C. with water at 15°C. as the standard was 0.9075. The optical rotation could not be taken directly because of the high degree of color of the oil. To obtain an approximate valuation of the effect on the plane of polarized light one volume of the oil was dissolved in four volumes of 95 per cent alcohol. This solution of the oil showed a levorotation of 7.36° in a 100 mm. tube. The index of refraction was 1.4990 at 24°C. The specific refraction is 0.323.

CHEMICAL CONSTANTS OF THE OIL

The following data were obtained: acid number, 0.508; saponification number, 28.7; saponification number after acetylation, 61.92. On account of the small amount of material at hand the identification of the acid and the alcohol was not undertaken.

TEST FOR PHENOL

Five c. c. of the oil shaken with 4 per cent aqueous solution of caustic potash diminished about 0.2 c. c. in volume, corresponding to about 4 per cent by volume of phenol. The oil emulsified very readily and the emulsion separated very slowly. A sharp line between the oily layer and the aqueous alkaline layer could not be obtained, but the minimum amount of diminution could safely be put at 0.2 c. c.

With choloform and KOH two or three drops of the oil gave a distinct pink color after standing a few minutes, a good indication of the presence of thymol or carvacrol or both. However, there seems to be no record of an oil from the family *Compositae* which contains either of these phenols. Whether the presence of this phenol is due to an impurity or not will have to be ascertained by the distillation and testing of other oils.

The phenol was removed from the total amount of oil by shaking with 4 per cent aqueous KOH, acidifying and extracting with ether. After evaporation of the ether there remained a reddish-brown mixture, having a bitterish pungent taste. With ferric chloride solution this residue gave a purple color, indicating the presence of salicyelic acid.

SAPONIFICATION AND FRACTIONATION OF THE OIL

The oil from which the phenol had been removed was next boiled on a water bath with an excess of alcoholic KOH for three quarters of an hour. The greater portion of the alcohol was then distilled off on a water bath and the alcoholic distillate added to a large volume of water. A very turbid mixture was obtained from which there separated very slowly a few c. c. of an oil having a slight odor of the original oil and also an odor suggesting pinene.

The alkaline liquid remaining in the flask after distilling off the alcohol was mixed with a relatively large volume of water, the oil which separated was removed and washed with water until free from alkali. It was then distilled with steam and collected in five fractions.

The physical constants of these fractions were as follows:

No. of Fraction.	$d_{15}^{25^{\circ}}$	$n_{D_{20}^{\circ}}$	α_D for 100 mm. tube.	$\frac{n^2-1}{n^2+2} \cdot \frac{1}{d}$
1.....	0.9089	1.4992	-37.05	0.323
2.....		1.5010	-31.85	
3.....	0.9110	1.5014	-31.07	0.323
4.....		1.5019	-24.85	
5.....	0.9196	1.5025	-19.35	0.321

DISTILLATION UNDER ATMOSPHERIC PRESSURE

A few c. c. each of fractions I, II, and V were distilled under ordinary pressure. The greater part of each fraction distilled as follows:

Fraction I between 240° and 275°C .

Fraction II between 250° and 280°C .

Fraction V between 255° and 280°C .

In all cases there was some decomposition and the distillate from each fraction possessed an empyreumatic odor.

The specific gravity, the index of refraction and the boiling temperature indicate that the oil is composed largely of one or more sesquiterpenes, and since the boiling temperature is not constant it is evidently a mixture.

If they were sesquiterpenes of the specific gravity indicated in the above table, fractions I, III, and V would have the molecular refractions 65.982, 65.892, and 65.484 respectively and according to both their specific gravities and their molecular refractions they would belong to the bicyclic sesquiterpenes with two double bonds as shown by the following table given in *G. H. K.* Vol. I, page 327.

	Mol. Refrac. (Calc.)	$d_{15}^{25^{\circ}}$
Aliphatic Sesquiterpenes	69.50	about 0.86
Monocyclic Sesquiterpenes	67.76	about 0.875-0.890
Bicyclic Sesquiterpenes	66.15	about 0.900-0.920
Tricyclic Sesquiterpenes	64.45	about 0.930-0.940

Fraction No. 1 was treated according to the method given in *G. H. K.* vol. 1,334, for the preparation of caryophyllene nitro-site but the result was negative.

A CHEMICAL STUDY OF WISCONSIN WORMWOOD OIL

Although wormwood has been distilled in Wisconsin for more than half a century, the oil produced in this state has never been subjected to a systematic control. Hence, it is impossible at the present time to state within what limits the physical and chemical constants of the Wisconsin oil may be expected to fluctuate. Neither is it known what allowances should be made for slight admixtures of other volatile oil producing plants in the still when a fair degree of care is exercised in the elimination of weeds. The same ignorance prevails with regard to the influences exercised by climatic conditions and methods of cultivation, the degree of development attained by the plant at the time it is harvested, the treatment which the plant receives between cutting and distillation, the length of distillation, the re-use of the aqueous distillate, the effect of the destruction of leaves by insects, etc.

It will require years of careful study to find satisfactory answers to some of these problems. When once found, it should be possible to give material aid to those engaged in this industry and to make the Wisconsin oil the best in the market. Already it would seem that a partial answer at least can be given as to the better methods of cultivation. It appears that the cultivation in rows yields a better oil as well as a better herb than the meadow cultivation in which cattle are permitted to do such weeding as they will. Whether the cultivation in rows will prove equally profitable remains to be seen.

In order to secure the necessary data that might enable the Station to answer questions in the future with regard to perplexing problems, it seemed highly desirable to begin at once with the investigation of samples of Wisconsin oils distilled during the season of 1913. For this purpose the farms in Sauk county that are at present engaged in the raising of wormwood

and the distillation of the oil were re-visited by two members of the Station and arrangements were made for procuring average one pound samples of the 1913 distillate. These are numbered 1, 2, and 5 in the list given below. Samples 3 and 4 consisted approximately of one-half pound each and were brought to the Station by Mr. Leander Drew for the purpose of having them compared. No. 6 is the oil distilled by Mr. G. A. Russell, Government Expert at the Northern Station for the Cultivation of Medicinal Plants, a co-operative experiment between the U. S. Department of Agriculture and the University of Wisconsin.

Inasmuch as the wormwood industry is without doubt the oldest medicinal plant industry in the state, and, moreover, one that has proven its capacity to exist, no further justification is required for giving special attention to it at the very beginning of the Station's activity. It is to be hoped that the investigation begun may be continued uninterruptedly for a number of years, for the results of a series of years will be necessary in order to secure the data that are needed for a better understanding of the subject.

SAMPLES OF 1913 OIL

- No. 1. One lb. of oil distilled by Harry Drew, Sauk Co.
- No. 2. One lb. of oil distilled by Gannon, Sauk Co.
- No. 3. One-half lb. of oil submitted by Leander Drew, Sauk Co.
- No. 4. One-half lb. of oil submitted by Leander Drew, Sauk Co.
- No. 5. One-half lb. of oil distilled by Henry Haggard, Sauk Co.
- No. 6. Oil distilled by G. A. Russel on the Northern Station, Madison, Dane Co.

ODOR AND COLOR

In odor and color these oils show very little difference, number six probably having the most pleasant odor. The color of all the samples was a very deep brown. It was, therefore, impossible to determine the optical rotation.

On exposure to the air in thin layers these oils soon became greenish-blue and finally deep blue. From observations made when the oils were rectified by steam distillation it is concluded

that the blue compound results from the high boiling constituents of the oil. Light does not seem to have any influence in the formation of this blue compound.

SPECIFIC GRAVITY

The specific gravity of each sample was determined by means of the pycnometer and that of five samples also by means of the Mohr-Westphal Hydrostatic balance with the following results:

Sample of Oil	$d \frac{25^\circ}{25^\circ}$ by pycnometer	$d \frac{25^\circ}{15^\circ}$ by hydrostatic balance
1.....	0.9350	0.9335
2.....	0.9230	0.9215
3.....	0.9350	0.9340
4.....	0.9231	
5.....	0.9263	0.9225
6.....	0.9364	0.9355

The specific gravity given in Gildemeister and Hoffmann, *The Volatile Oils*, is from 0.9250 to 0.9550. From the preceding data it will be seen that samples No. 2 and 4 are unusually low in specific gravity.

INDEX OF REFRACTION

The index of refraction was determined by means of the Abbé Refractometer at the temperature of 19°C.

No. of samples.	$D_{n_{19^\circ}}$
1	1.4712
2	1.4705
3	1.4712
4	1.4675
5	1.4721
6	1.4725

SOLUBILITY IN 80 P. C. ALCOHOL

The high degree of color makes it somewhat difficult to determine the solubility of these oils accurately. The solubility of all these samples seemed to be practically the same, one c. c. of each requiring between 0.7 c. c. and 0.8 c. c. of 80 p. c. alcohol to form a clear liquid. Gildemeister and Hoffmann state that the oil forms a clear solution with 2 to 4 parts of 80 p. c. alcohol.

SAPONIFICATION OF THE OILS

Two determinations were made in each case. Between four and six grams of the oil were heated on a boiling water bath with 50 c. c. of an approximately semi-normal alcoholic potassium hydroxide solution. The time of heating was one half hour and one hour respectively. The alkaline mixture was then diluted with distilled water so that the aqueous portion would measure 500 c. c. This solution was then titrated in 100 c. c. portions, using approximately semi-normal sulphuric acid. Phenolphthalein was used as indicator. The solutions were yellowish to yellowish-brown in color. In some cases the solution from the mixture which had been heated a half hour was darker than that from the mixture which had been heated an hour although the conditions of heating were apparently the same. The 100 c. c. portions of solutions were placed side by side on a white background and acid run in until the color was of the same shade as that of a portion of the solution to which no indicator had been added. Usually a small drop of the acid was sufficient to remove the pink color. This solution was then used as a color standard in titrating the last portion. The results are given in the following table:

Sample of oil	Gms. of oil used	C.C. of $\frac{N}{2}$ alc. KOH used	Length of time heated	C. C. $\frac{N}{2}$ H_2SO_4 required for 100 c.c. of solution	Saponification number	Amount of thujyl alcohol	Amount of ester expressed as thujyl acetate
1 a	4.7376	56.7	half hour	7.94	100.4	27.61	35.14
1 b	5.2484	56.7	one "	7.6	99.7	27.42	34.89
2 a	5.3329	56.7	half "	8.45	75.8	20.84	26.53
2 b	5.4642	56.7	one "	8.46	73.78	20.3	25.83
3 a	5.1840	56.7	half "	7.63	100.19	27.55	35.07
3 b	5.3092	56.7	one "	7.64	97.56	26.84	34.16
4 a	5.2851	56.47	half "	7.61	97.58	26.84	34.16
4 b	4.9747	56.62	one "	7.85	97.65	26.85	34.18
5 a	5.6123	56.81	half "	7.5	96.29	26.48	33.70
5 b	5.6400	56.81	one "	7.56	94.32	25.93	33.00
6 a	5.1156	56.81	half "	8.08	89.61	24.64	31.36
6 b	5.5468	56.81	one "	7.6	94.9	26.10	33.21

RECTIFICATION OF THE OILS

100 c. c. each of samples Nos. 1, 2, 3, and 6 were subjected to steam distillation. From No. 1 about 95 c. c. of oil were recovered. 94 c. c. were obtained from each of Nos. 2 and 3. From No. 6 a smaller amount of oil was obtained, namely 88 c. c. In each case the distillation was continued until practically no oil separated from the distillate. The color of the first few cubic centimeters of oil carried over in each case was very light brown but as the distillation proceeded the color deepened so that the color of the rectified oil was only slightly less pronounced than that of the original oil. In each case the last portion of oil which passed over was very highly colored—almost black. A drop of this on exposure to the air soon became blue.

SPECIFIC GRAVITY OF THE RECTIFIED OILS

The specific gravity was determined by means of the Mohr-Westphal Hydrostatic balance, the temperature of the oils being

25°C. For the purpose of comparison the sp. gr. of the original oils and of the corresponding rectified oils is given in the following table.

Sample of oil	Original oil	Rectified oil.
1	0.9335	0.9312
2	0.9215	0.9190
3	0.9340	0.9303
6	0.9355	0.9200

SAPONIFICATION OF THE RECTIFIED OILS

The same process was used here as in the saponification of the original oils. One determination was made in each case and the length of time of heating was a half-hour. The following saponification numbers were obtained. For the sake of ready comparison, the saponification values of the original oils are included in the tabulation.

Sap. No. of rectif. oil	Sap. No. of orig. oil
Sample No. 1.—105.8	100.4
Sample No. 2.—79.11	75.8
Sample No. 3.—98.97	100.19
Sample No. 6.—88.57	89.61

ACETYLATION OF THE OILS

10 c. c. of each sample of oil was acetylated according to the directions given in Gildemeister and Hoffmann, *The Volatile Oils*, p. 195. The length of time of heating was one hour.

SAPONIFICATION OF THE ACETYLATED OILS

The same procedure was followed here as previously described except that 100 c. c. of the alcoholic potassium hydroxide solution were used for each determination. The length of time of heating was in each case one hour. The saponification numbers, the corresponding amount of alcohol as $C_{10}H_{18}O$, the amount of combined alcohol present as acetate, the amount of free alcohol

in the oils, and the amount of acetate present are given in the following table:

Sample of oil.	Saponification number.	Corresponding amount of alcohol.	Alcohol combined as acetate.	Amount of free alcohol in oil=thujyl alc.	Amount of acetate present=thujyl acetate
		Per cent.	Per cent.	Per cent.	Per cent.
1.....	157.13	48.97	27.42	21.55	34.89
2.....	113.93	34.25	20.30	13.95	28.83
3.....	134.29	41.07	26.84	14.23	34.16
4.....	118.44	35.74	26.35	8.89	34.17
5.....	129.53	29.45	25.93	13.52	33.00
6.....	125.41	28.06	26.10	11.96	33.12

FRACTIONATION OF THE OILS

Sample No. 1. The distillation was carried out in a Ladenburg flask, at ordinary pressure and over a bare flame. The amount of oil used was 50 c. c. The rate of distillation was about 60 drops per minute. The distillate was collected in fractions of five cubic centimeters each. In the case of this oil a small amount of liquid, probably 0.5 c. c., came over near 100°C but the mercury rose rapidly to about 195°. Eight fractions were collected. The first fraction was of a light yellowish brown color the intensity of color being too great to permit taking the optical rotation with any degree of accuracy. It was seen, however, to be dextrorotatory. About 0.5 c. c., dissolved in 3.5 c. c. of 95 p. c. alcohol, gave a rotation in a 50 mm. tube of about +3.1°. Fraction No. 2 was of about the same shade of color as fraction No. 1, but the highest fractions were quite highly colored. The temperature at which the fractions came over was as follows:

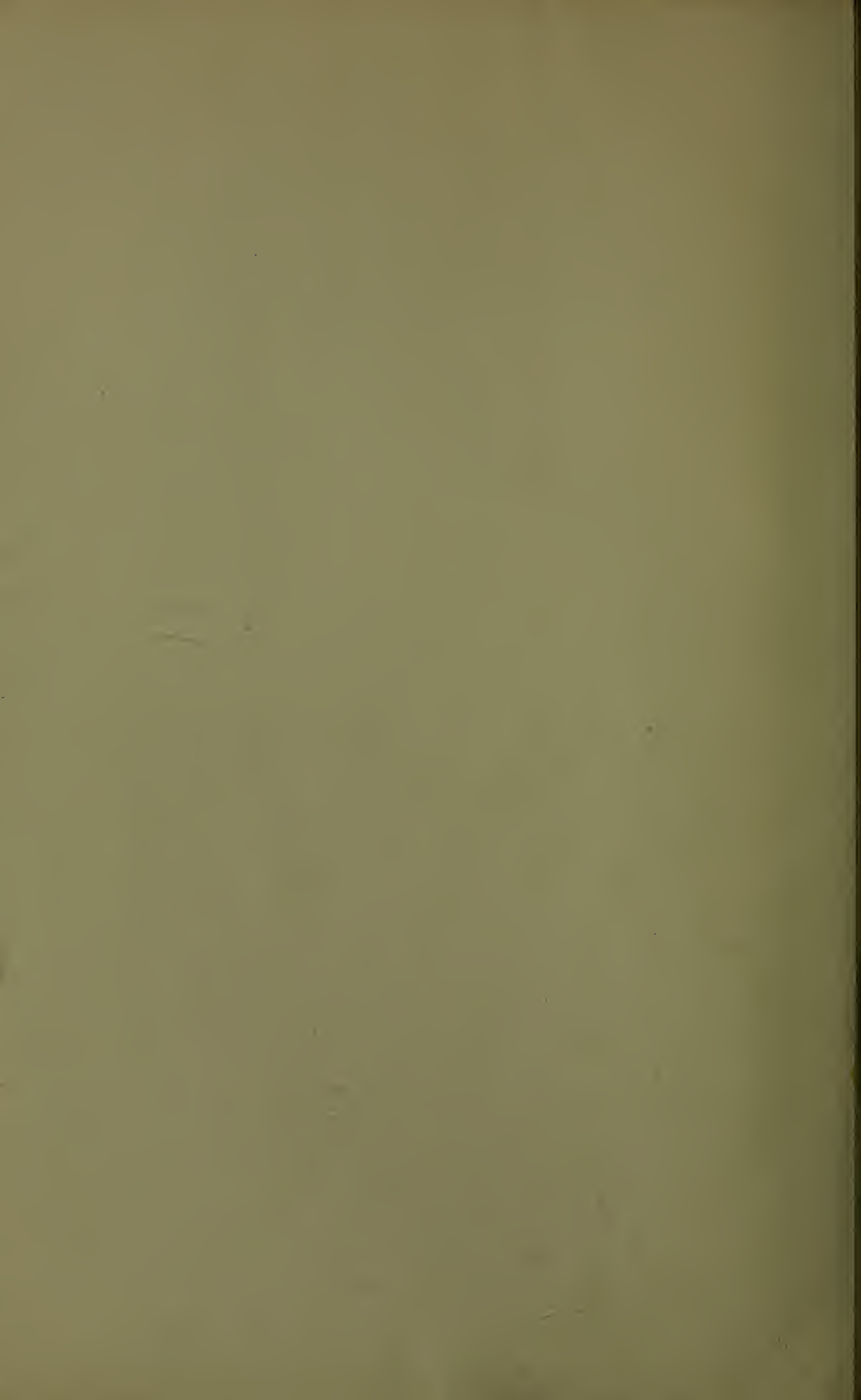
Fraction No. 1	195°-203°C
Fraction No. 2	203°-206°C
Fraction No. 3	206°-207°C
Fraction No. 4	207°-208°C
Fraction No. 5	208°-210°C
Fraction No. 6	210°-214°C
Fraction No. 7	214°-219°C
Fraction No. 8	219°-232°C

After standing 24 hours in a tightly stoppered vial, fraction No. 2 had become light greenish in color. The other fractions appeared not to have changed in color. After standing several days in small stoppered vials all the fractions had become greenish blue, the depth of blue gradually increasing with increase in boiling point.

Sample No. 2. In this case there appeared to be more water present. Quite a number of drops of water condensed in the neck of the flask. Practically no liquid passed over below 190°. Most of the first fraction came over between 195° and 202°. The appearance of the fractions was just like that of the corresponding fractions from oil No. 1. The temperature at which the fractions distilled was as follows:

Fraction No. 1	195°-202°
Fraction No. 2	202°-205°
Fraction No. 3	205°-207°
Fraction No. 4	207°-210°
Fraction No. 5	210°-213°
Fraction No. 6	213°-217°
Fraction No. 7	217°-223°
Fraction No. 8	223°-243°

After standing 24 hours in a small stoppered vial fractions 2 and 3 had changed to a light green color. After standing several days these fractions were of about the same color as the corresponding fractions from sample No. 1.



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